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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor: Biel, Merrill A.

Serial No: 0

Filing Date: February 26, 2000

Title: Photodynamic Therapy Utilizing a Solution of
Photosensitizing Compound and Surfactant

PATENT

Art Group Unit:

Examiner:

22272-14

TRANSMITTAL

ASSISTANT COMMISSIONER OF PATENTS
Washington, D.C. 20231

Enclosed for filing please find the following:

1. Continuation-in-Part Application (10 sheets of text, and 1 sheet of drawings);
2. Declaration, Power of Attorney, and Petition (3 pages);
3. Verified Statement (Declaration) Claiming Small Entity Status - Independent Inventor (2 pages);
4. Check in the amount of \$435.00 for payment of utility filing fee; and
5. Return Receipt Postcard.

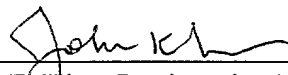
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Please direct any questions or comments to John F. Klos at (612) 896-1520.

Respectfully submitted,

Date: February 26, 2000

By:


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I hereby certify that this paper and any papers referred to herein are being deposited with the U.S. Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above addressed, Assistant Commissioner of Patents, Washington, D.C. 20231.

John F. Klos


Signature

0559352.01

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor:	BIEL, Merrill A.)	PATENT
)	
Serial No:)	Art Group Unit:
)	
Filing Date:	February 25, 2000)	Examiner:
)	
Title:	Photodynamic Therapy Utilizing a)	VERIFIED STATEMENT
	Solution of Photosensitizing)	
	Compound and Surfactant)	

Honorable Commissioner of Patents and Trademarks
c/o ASSISTANT COMMISSIONER OF PATENTS
Washington, D.C. 20231

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS
(37 CFR 1.9(f) AND 1.27 (b)) - INDEPENDENT INVENTOR

As a below named inventor, I hereby declare that I qualify as an independent inventor as defined in 37 CFR §1.9(c) for purposes of paying reduced fees under Sections 41(a) and (b) of Title 35, United States Code, to the Patent and Trademark Office with regard to the invention Photodynamic Therapy Utilizing a Solution of Photosensitizing Compound and Surfactant described in the specification filed herewith.

I have not assigned, granted, conveyed or licensed and am under no obligation under contract or law to assign, grant, convey, or license, any rights in the invention to any person who could not be classified as an independent inventor under 37 CFR §1.9(c) if that person had made the invention, or to any concern which would not qualify as a small business concern under 37 CFR §1.9(d) or a non-profit organization under 37 CFR §1.9(e).

Each person, concern or organization to which I have assigned, granted, conveyed or licensed or am under an obligation under contract or law to assign, grant, convey, or license any rights in the invention listed below: Advanced Photodynamic Technologies, Inc.

NAME Advanced Photodynamic Technologies, Inc.

ADDRESS 2715 4th Street, S.E. Suite 70, Minneapolis, MN 55414

☐ Individual ☒ Small Business Concern ☐ Nonprofit Organization

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR §1.28(b)).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18, United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Name of inventor: Merrill A. Biel

Inventor's Signature: _____

Date: _____

Merrill A. Biel
2/25/00

PHOTODYNAMIC THERAPY UTILIZING A SOLUTION OF PHOTSENSITIZING COMPOUND AND SURFACTANT

Related Application

This application is a continuation-in-part application based on prior copending application Ser. No. 09/139,866 filed August 25, 1998, the benefit of the priority date is hereby claimed pursuant to 35 U.S.C. §120.

Field of the Invention

The invention relates to a photodynamic therapy (PDT), and more particularly to a photodynamic therapy utilizing a combined solution of a photosensitizing compound and surfactant compound. Still more specifically, the invention relates to photodynamic inactivation of bacteria, fungal, and viral wound infections and sterilization of tissue using methylene blue or toluidene blue and a surfactant, such as polymyxin B or SDS. Additionally, the invention relates to photodynamic eradication of cancer cells, such as present within a tumor, by PDT in conjunction with an administration of a solution including a photodynamic sensitizing compound and a surfactant. The present invention advantageously uses light energy in combination with a photosensitizing agent and a surfactant solution to treat both invitro and invivo pathogens, including cancer cells and microbiological pathogens.

Background of the Invention

Abnormal cells in the body are known to selectively absorb certain dyes delivered to a treatment site to a more pronounced extent than surrounding tissue. Once presensitized, the cancerous or abnormal cells can be destroyed by irradiation with light of an appropriate wavelength corresponding to an absorbing wavelength of the dye, with minimal damage to surrounding normal tissue. This procedure, which is known as photodynamic therapy (PDT), has been clinically used to treat metastatic breast cancer, bladder cancer, head and neck cancers, and other types of malignant tumors.

Summary of the Invention

The present invention provides a method of photoeradication of cells at a tissue site, such as during an invitro or invivo disinfection procedure, or during a sterilization procedure, or for cancer cell destruction. The method utilizes a combined solution of a photosensitizing dye and a surfactant compound. The invention provides a method of disposing the combined solution at or near the tissue site and subsequently irradiating the tissues site with laser light at a wavelength absorbed by the photosensitizing dye.

The invention relates to use of a photosensitizing agent, such as methylene blue or toluidene blue, in combination with a surfactant compound, such as polymyxin B or SDS, in a PDT treatment protocol against bacterial or fungal infections or for cancer cell photoeradication. A treatment device is configured to deliver light energy to the area of infection at wavelengths ranging from about 450 nm to about 850 nm; provide a dosage rate ranging from about 0 to about 150 mw/cm²; and provide a light dose ranging from 0 to about 300 J/cm².

The use of a photosensitizer, e.g., methylene blue or toluidene blue, combined with a surfactant compound, such as SDS or polymyxin B, in a photodynamic therapy advantageously acts as a broad spectrum antimicrobial, i.e., antibacterial, antiviral, and antifungal agent. The dye/surfactant combination and PDT may be used, for example, before a surgical operation. The present invention advantageously results in the destruction of gram positive and gram negative bacteria and fungus. Importantly, the present invention acts to destroy antibiotic resistant bacteria as it utilizes a different destruction mechanism than antibiotics.

The invention relates to a method of treating an infection including identifying an in-vivo area of infection; applying a concentration including a photosensitizing dye, such as methylene blue or toluidene blue, and a surfactant, such as polymyxin B or SDS, to the area of infection; and exposing the area of infection with a light having a light wavelength, light dosage and a light dosage rate. The light wavelength may range from about 610 nm to about 680 nm. The light dosage may range from about 10 J/cm² to about 60 J/cm². The light dosage rate may range from about 50 mw/cm² to about 150 mw/cm². The wavelength may range from about 630 nm to about 664 nm. The concentration may range from about 10 µg/ml to about 500 µg/ml. The area of infection may include gram positive and gram negative bacteria, fungus, or virus including,

but not limited to, at least one of staphylococcus, candida albicans, escherichia coli, enterococcus, streptococcus, pseudomonas aeruginosa, hemophilus influenzae, or clostridia.

The invention also relates to a treatment kit including a volume of a concentration including at least a combination of methylene blue or toluidene blue and polymyxin B or SDS. The concentration ranges from about 3 $\mu\text{g/ml}$ to about 500 $\mu\text{g/ml}$. Also included is a light emitting treatment device. The light emitting treatment device is configured to emit at wavelengths ranging from about 450nm to about 850nm; provide a dosage rate ranging from about 0 to about 150 mw/cm^2 ; and provide a light dose ranging from 0 to about 300 J/cm^2 .

The invention also relates to a method of treating an infection, an invitro or invivo sterilization procedure, or photoeradication of cancer cells, including providing one or more cells; disposing a concentration of combined dye/surfactant on the one or more cells; applying a light having a wavelength ranging from about 450nm to about 850nm; a dosage rate ranging from about 0 to about 150 mw/cm^2 ; and a light dose ranging from 0 to about 300 J/cm^2 to the one or more cells wherein the combination of light and dye is adapted to cause photodestruction of the one or more cells. The one or more cells may be a bacteria, virus, a fungus, or cancer cells. The one or more cells may be gram positive or gram negative. The dye may be methylene blue, toluidene blue, or a combination thereof. The dye may be monomeric or dimeric.

Still other objects and advantages of the present invention and methods of construction of the same will become readily apparent to those skilled in the art from the following detailed description, wherein only the preferred embodiments are shown and described, simply by way of illustration of the best mode contemplated of carrying out the invention. As will be realized, the invention is capable of other and different embodiments and methods of construction, and its several details are capable of modification in various obvious respects, all without departing from the invention. Accordingly, the drawing and description are to be regarded as illustrative in nature, and not as restrictive.

Brief Descriptions of the Drawings

Fig. 1 is a table of results of photodynamic photoeradication using a combined solution of methylene blue and the surfactant, SDS.

Detailed Description of the Invention

In accordance with the invention, a photodynamic therapy is provided utilizing a photosensitizing agent such as methylene blue or toluidene blue in combination with a surface acting agent or 'surfactant' and a light emitting treatment device such as a light wand, light patch, light pad or shaped light-emitting article such as a mouthpiece to illuminate the site.

The present invention may be used in conjunction with or in relation to inventions disclosed in the following applications of the applicant, including:

Method of Enhancing Photodynamic Therapy by Administering an Immunologic

Adjuvant, U.S. pat. app. Ser. No. 09/139,861, pending;

Expandable Treatment Device for Photodynamic Therapy and Method of Using

Same, U.S. pat. app. Ser. No. 09/239,353, pending;

Spatial Orientation and Light Sources and Method of Using Same for Medical

Diagnosis and Photodynamic Therapy, U.S. pat. app. Ser. No. 09/139,862, now

U.S. Pat. No. _____; and

Treatment Device for Topical Photodynamic Therapy and Method of Making Same,

PCT/US9817589, WO 99/10046, pending.

All documents within these applications are herein incorporated by reference in their entireties for all purposes.

Photosensitizing dyes and compounds for use in accordance with the present invention are generally non-toxic to the target microbes and surrounding tissues at concentrations envisaged. However, there is no particular requirement that the photosensitizers should be non-toxic to the microbes. Particular photosensitizers which may be used in accordance with the invention include dyes and compounds such as methylene blue and toluidene blue.

Surface acting agents, or surfactants, are defined as substances which alter the energy relationship at interfaces. Among the manifestations of these altered energy relationships is the lowering of surface or interfacial tensions. Compounds displaying surface activity are characterized by an appropriate structural balance between one or more water-attracting groups and one or more water-repellent groups. Surfactants characterized by having two different moieties, one polar and the other nonpolar. The polar moiety is referred to as hydrophilic or

lipophobic, and the nonpolar as hydrophobic or lipophilic. The electrical charge on the hydrophilic portion of a surface acting agent may serve as a convenient basis of classification of these compounds. Surface active agents have been classified as : Anionic, Cationic, Non-Ionic, and Amphoteric. Surfactants are known to affect the permeability of cell membranes. The ability of surfactants to become oriented between lipid and protein films is thought to produce a disorientation of the lipoprotein membrane of microorganisms, so that it no longer functions as an effective osmotic barrier. The polymyxins, colistin, and the polyene antifungal agents nystatin and amphotericin are surfactants, as is sodium dodecyl sulfate (SDS).

A photosensitizing dye compounds such as methylene blue or toluidene blue may be used in combination with surfactants, such as SDS and polymyxin B, and activated by light energy at approximately 630-660 nm wavelengths to provide broad spectrum antibiotic activity for destroying both gram positive and gram-negative bacteria, fungus, virus and cancer cells. The dye compound and surfactant may be combined in solution and administered to a site to be treated. Solution administration may include topical application, or intravenous, subcutaneous, intratumor, or peritumoral injection. Additional administration approaches may also be practicable. An intratumoral injection of the solution may be advantageous for photoeradication of tumor cells.

A particular treatment protocol utilizing the photosensitizer methylene blue and SDS concentrations from 0.001% to 0.01 % has been identified as advantageous in the destruction of certain microorganisms, such as candida albicans, escherechia coli, pseudomonas aeruginosa, staphylococcus aureus, and streptococcus pneumoniae.

Reference may be made to FIG. 1, which illustrates a schedule of results for an investigation according to the present invention of photoeradication using methylene blue mediated PDT and the surfactant, SDS, at a wavelength of approximately 664 nm. The laser used was a diode laser with a power output of 1000 mW. Methylene blue concentrations range from 5 to 100 µg/ml. SDS concentrations range from 0.003 to 0.01 %. Light power ranges from 0.127 to 0.3 watts. The combined solution was topically applied at the cell site. Light dosage rates range from 75 to 100 mW/cm². Light dosages range from 15 to 60 J/cm². A qualitative colony count relates particular colony counts to an associated score. For instance, a qualitative

colony count of 5 would yield a score of '1', while a colony count of 151 would have an associated score of '3'. The results of this investigation demonstrated that the solution including a surfactant and a photosensitising agent can sensitize several species of bacteria to killing by diode laser irradiation.

A laser light source may be used to practice the present invention. A variety of light sources are currently available, and the selection of a particular laser light source for implementing the PDT would readily be appreciated by those skilled in the relevant arts. The laser source will be selected having regard to the choice of wavelength, beam diameter, exposure time and sensitivity of the microbes to the laser/photosensitizer/surfactant combination.

The above described embodiments of the invention are merely descriptive of its principles and are not to be considered limiting. Further modifications of the invention herein disclosed will occur to those skilled in the respective arts and all such modifications are deemed to be within the scope of the invention as defined by the following claims.

Claims

Claim:

1. A method of treating photoeradication of cells comprising the steps of:

identifying an area of infection or an area of sterilization or an area of cancer cell activity;

applying a concentration including a combination of a surfactant and a photosensitizing dye compound to the area of infection or the area of sterilization or the area of cancer cell activity; and

exposing the area of infection or the area of sterilization or the area of cancer cell activity with a light having a light wavelength, light dosage and a light dosage rate.
2. The method of photoeradication of cells of claim 1 wherein the light wavelength ranges from about 400 nm to about 800 nm, the light dosage ranges from about 10 J/cm² to about 100 J/cm² and the light dosage rate ranges from about 50 mw/cm² to about 200 mw/cm².
3. The method of photoeradication of cells of claim 1 wherein the wavelength ranges from about 600 nm to about 700 nm.
4. The method of photoeradication of cells of claim 1 wherein the photosensitizing dye is methylene blue.
5. The method of photoeradication of cells of claim 4 wherein a concentration range of the methylene blue is from about 5 µg/ml to about 100 µg/ml.
6. The method of photoeradication of cells of claim 1 wherein the application of the concentration is a topical application.
7. The method of photoeradication of cells of claim of claim 1 wherein the surfactant is either polymixin B or SDS, or combinations thereof.
8. The method of photoeradication of cells of claim 1 wherein the application of the concentration is achieved via one or more of the group containing an intravenous injection, an intratumor injection, a subcutaneous injection, and a pertumoral injection.

9. A photodynamic therapy treatment kit comprising:

a volume of a concentration including a combination of a surfactant and a photosensitizing dye compound; and

a light emitting treatment device configured to emit light at wavelengths ranging from about 450nm to about 850nm; to provide a dosage rate ranging from about 0 to about 150 mw/cm² and a light dose ranging from 0 to about 300 J/cm².

10. A method of treatment comprising:

providing one or more cells;

disposing a concentration in proximity to the one or more cells, said concentration including a combination of a surfactant and a photosensitizing dye compound on the one or more cells;

applying a light in proximity to the one or more cells, said light having a wavelength ranging from about 450nm to about 850nm; a dosage rate ranging from about 0 to about 150 mw/cm²; and a light dose ranging from 0 to about 300 J/cm², wherein the combination of the light and the surfactant and the dye compound is adapted to cause intracellular enzyme deactivation of the one or more cells.

11. The method of treatment of claim 10 wherein the step of disposing the concentration is achieved via one or more of the group containing: an intratumor injection, an intravenous injection, a topical application, and a peritumoral injection.

12. The method of treatment of claim 10 wherein the one or more cells include at least one of a bacteria, a fungus, a virus, or a cancer cell.

13. The method of treatment of claim 10 wherein the one or more cells are gram positive or gram negative.

14. The method of treatment of claim 10 wherein the dye compound is at least one of methylene blue, toluidene blue, or combinations thereof.

15. The method of treatment of claim 10 wherein the dye compound is monomeric or dimeric.
16. The method of treatment of claim 10 wherein the step of providing one or more cells is associated with a sterilization procedure.
17. The method of treatment of claim 10 wherein the step of providing one or more cells is associated with treatment of an infection at a tissue site.
18. The method of treatment of claim 10 wherein the step of providing one or more cells includes providing one or more of a fungus or a virus or a cancer cell.

Abstract

The invention relates to a method of photoeradication of cells at a tissue site, such as an infection or sterilization site or cancer cell activity site, including applying a solution such as methylene blue, toluidene blue, polymyxin B, SDS, or other surfactants and combinations thereof to the tissue site and exposing the tissue site with a light having a light wavelength and light dosage and a light dosage rate. The solution may have a concentration of methylene blue between 5 $\mu\text{g/ml}$ to about 100 $\mu\text{g/ml}$. The wavelength may range from about 610 nm to about 670 nm. The light dosage may range from about 0 J/cm^2 to about 200 J/cm^2 . The light dosage rate may range from about 0 mw/cm^2 to about 150 mw/cm^2 . Treatable organisms include candida albicans, escherechia coli, pseudomonas aeruginosa, staphylococcus aureus, streptococcus pneumoniae, and clostridia.

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Photoirradiation using Methylene Blue mediated PDT & the Surfactant SDS									
Qualitative Colony Count									
Organism (9x10 ⁹ /mL)	Meth. Blue (µg/mL)	SDS %	Total Power (W)	Light Dose Rate (mW/cm ²)	Light Dose (J/cm ²)	No Light	Single		
							Light Dose	Light Dose	
<i>Candida albicans</i>	100	0.0075	0.3	100	60	4	0	0	0
	100	0.01	0.3	100	60	4	1	0	0
	25	0.003	0.127	100	40	4	0	0	n/a
	100	0.003	0.23	150	60	4	4	1	1
	30	0.003	0.127	100	40	4	0	0	n/a
<i>Escherchia coli</i>	30	0.005	0.127	100	40	4	0	0	n/a
	40	0.003	0.127	100	40	4	0	0	n/a
	40	0.005	0.127	100	40	4	0	0	n/a
	3	0.003	0.225	75	15	4	0	0	n/a
<i>Streptococcus pneumoniae</i>	3	0.005	0.225	75	15	4	0	0	n/a
	5	0.003	0.225	75	15	4	0	0	n/a
	5	0.005	0.225	75	15	4	0	0	n/a
	5	0.0075	0.225	75	15	4	0	0	n/a
	5	0.0075	0.225	75	15	4	0	0	n/a

FIG. 1

DECLARATION, POWER OF ATTORNEY, AND PETITION

As the below-named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated immediately below next to my name;

Full name of inventor: Merrill A. Biel

Residence (City/State): Minneapolis, Minnesota

Mailing Address: 2715 4th Street Southeast, Suite 70

Citizenship: United States

I believe I am an original, first, and sole inventor (if only one name appears above) or an original, first, and joint inventor (if plural names appear above) of the subject matter which is claimed and for which a patent is sought on the invention entitled Photodynamic Therapy Utilizing a Solution of Photosensitizing Compound and Surfactant, the specification of which is attached hereto;

I state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to herein;

I acknowledge the duty to disclose information material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a), including any public use, sale, or offer for sale of the disclosed invention occurring more than one year prior to the filing date of said prior application.

I hereby claim priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed pursuant to §119 (a) or of any provisional patent application(s) listed pursuant to §119(e) and have identified any such application(s) for patent or inventor's certificate having a filing date before that of the application on which priority is claimed: **none**

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the

first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

U.S. pat. app. Ser. No. 09/139,866, filed August 25, 1998, and copending

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

And I hereby appoint, jointly and severally, Frederick W. Niebuhr, Registration No. 27,717, John F. Klos, Registration No. 37,162 and LARKIN, HOFFMAN, DALY & LINDGREN, LTD., 1500 Norwest Center, 7900 Xerxes Avenue South, Bloomington, Minnesota 55431-3333, my attorneys with full power of substitution and revocation to prosecute this application and to transact all business in the Patent and Trademark Office connected herewith and the general power of attorney to file and prosecute any foreign or International (PCT) application claiming the benefit of priority of this application, or any continuation, continuation-in-part, divisional, reexamination, or reissue thereof.

All communications shall be addressed to:

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Wherefore, I pray that Letters Patent be granted to me for the invention or discovery described and claimed in the above-referenced specification and claims, and I hereby subscribe my name to said specification and claims and to the foregoing declaration, power of attorney, and this petition.

Full name of inventor: Merrill A. Biel

Inventor's signature: 

Date: February 26, 2000.

0559359.01